

Serial No.: 10/057,467

Filed: January 22, 2002

IN THE SPECIFICATION:

Please replace the paragraph beginning at page 3, line 22, with the following rewritten paragraph:

— Figure 2. Creation of a library of random peptides in a retrovirus DNA construct by primed DNA synthesis (SEQ ID NOS:10-14).—

Please replace paragraph beginning at page 4, line 9, with the following rewritten paragraph:

– The introduced nucleic acids and resultant expression products are randomized, meaning that each nucleic acid and peptide consists of essentially random nucleotides and amino acids, respectively. The library may be fully random or biased, e.g., in nucleotide/residue frequency generally or per position. For example, a biased library may encode peptides for interactions with known classes of molecules, such as SH-3 domain proteins, as defined by peptides containing XXXPPXPXX (where X=randomized residues; SEQ ID NO:1). In other embodiments, the nucleotides or residues are randomized within a defined class, e.g., of hydrophobic amino acids, of purines, etc. In any event, where the ultimate expression product is a nucleic acid, at least 10, preferably at least 12, more preferably at least 15, most preferably at least 21 nucleotide positions need to be randomized; more if the randomization is less than perfect. Similarly, at least 5, preferably at least 6, more preferably at least 7 amino acid positions need to be randomized; again, more if the randomization is less than perfect.—

Please replace paragraph beginning at page 9, line 26, with the following rewritten paragraph:

Serial No.: 10/057,467

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— A scheme for generating a peptide library in the pBabe Puro vector is shown in Figure 2. Primers for PCR were synthesized, purified and deprotected according to standard protocols. Primer 1, complementary to polylinker sequences in the pBabe Puro retroviral construct, has the sequence 5' GCT TAG CAA GAT CTC TAC GGT GGA CCK NNK NNK NNK NNK NNK NNK NNK NNK NNC CCC ACT CCC ATG GTC CTA CGT ACC ACC ACA CTG GG 3' (SEQ ID NO:2). N represents any of the four bases; K is limited to G or T. Primer 2 has the sequence 5' GCT TAG CAA GAT CTG TGT GTC AGT TAG GGT GTG G 3' (SEQ ID NO:3) and is complementary to sequences within the pUC18 origin of replication. PCR was carried out for 8 rounds using primer 1, primer 2, Babe Puro as template, and a mixture of Taq DNA Polymerase (Promega) and Deep Vent DNA Polymerase (New England Biolabs) in a ratio of 128 Taq: 1 Deep Vent as described in Barnes (1994) Proc. Natl. Acad. Sci. USA, 91, pp. 2216-2220. The amplified PCR product was purified, digested with restriction enzymes Bgl II and Not I (Promega), purified again and ligated with the corresponding Bam HI-Not I fragment of pBabe Puro. After transformation the resulting library contained $\sim 2 \times 10^8$ clones, greater than 80% of which contained inserts. —

Please replace paragraph beginning at page 10, line 12, with the following rewritten paragraph:

— Oligonucleotides were synthesized and purified according to standard protocols. The "library" oligonucleotides have the sequence 5' CTG GAG AAC CAG GAC CAT GGG C (NNK)₁₀ GGG CCC CCT TAA ACC ATT AAA T 3' (SEQ ID

Serial No.: 10/057,467
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NO:4) or 5' CTG GAG AAC CAG GAC CAT GGG CNN KNN KNN KCC TCC
 CNN KCC TNN KNN KGG GCC CCC TTA AAC CAT TAA AT 3'(SEQ ID NO:5).

A third oligonucleotide ("constant"), complementary to the 3' ends of the library oligonucleotides, has the sequence 5'TCA TGC ATC CAA TTT AAT GGT TTA AG 3'(SEQ ID NO:6). As shown in Fig. 2, each library oligonucleotide is annealed to the constant oligonucleotide, converted to double stranded DNA with Sequenase (United States Biochemical) or Klenow (Promega), digested with restriction enzyme Bst XI (New England Biolabs), and purified and ligated with the appropriate Bst XI-digested retroviral construct. Transformation efficiencies are $\sim 2 \times 10^8$ clones per microgram of ligated DNA, greater than 90% of which contain an insert. A representative retrovirus is shown in Fig. 4; see also, retroviral vector with presentation construct nucleotide sequence (SEQ ID NO:7). —

Please delete the paragraph and subheading beginning at page 10, line 24 and continuing through page 13, line 7, inclusive.

Please replace paragraph beginning at page 13, line 25, with the following rewritten paragraph:

— In some embodiments of the invention, expression products are localized to, or preferentially concentrated in, different subcellular compartments within cells, e.g., by using appropriate addition of addressins to a peptide presentation construct, see, Figure 3. Addressins are available for a wide variety of subcellular locales including the nucleus, Golgi, mitochondria, plasma membranes, endoplasmic reticulum, secretory granules, secreted, cell surface (extracellular domain with

Serial No.: 10/057,467

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random), cell surface (intracellular domain random), etc. For example, many proteins whose functions require entry into the cell nucleus include nuclear localization signal (NLS) sequences: generally short, positively charged (basic) domains that serve to direct the entire protein in which they occur to the cell's nucleus. Numerous NLS amino acid sequences have been reported including single basic NLS's such as that of the SV40 (monkey virus) large T Antigen (Pro Lys Lys Lys Arg Lys Val (SEQ ID NO:8)), Kalderon (1984), et al., Cell, 39:499-509, and double basic NLS's exemplified by that of the Xenopus (African clawed toad) protein, nucleoplasmin (Ala Val Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys Leu Asp (SEQ ID NO:9)), Dingwall, et al., Cell, 30:449-458, 1982 and Dingwall, et al., J. Cell Biol., 107:641-849; 1988). Numerous localization studies have demonstrated that NLSs incorporated in synthetic peptides or grafted onto reporter proteins not normally targeted to the cell nucleus cause these peptides and reporter proteins to be concentrated in the nucleus. See, for example, Dingwall, and Laskey, Ann. Rev. Cell Biol., 2:367-390, 1986; Bonnerot, et al., Proc. Natl. Acad. Sci. USA, 84:6795-6799, 1987; Galileo, et al., Proc. Natl. Acad. Sci. USA, 87:458-462, 1990.—

On page 16, immediately preceding the claims, please insert the enclosed text entitled "SEQUENCE LISTING".

REMARKS

The specification and claims have been amended to include a Sequence Listing and proper reference to the sequences therein. Attached hereto is a marked-up version of the